Biological Response to Porcine Xenograft Implants: An Experimental Study in Rabbits

José Luis Calvo-Guirado, DDS, MS, PhD,* Gerardo Gómez-Moreno, DDS, MS, PhD,† Javier Guardia, DDS, PhD,‡ Antonio Ortiz-Ruiz, DDS, MS, MD, PhD,§ Adriano Piatelli, DDS, PhD, MS, MD,‖ Antonio Barone, DDS, PhD, MS,¶ José María Martínez-González, DDS, MS, MD, PhD,# Luis Meseguer-Olmo, MD, MS, PhD,** Laura López-Marí, DDS,†† and Cristina Barona Dorado, DDS, PhD, MS‡‡

Purpose: *The aim of this study was to evaluate the effect of a new porcine biomaterial and collagen paste in 20 New Zealand rabbits.*

Materials and Methods: *Forty implants using a porcine xenograft made up of 80% corticocancellous collagenated bone particles of ≤300 μm in size were placed in the proximal metaphyseal area of both tibiae. Four periods of time were formed: 1h, 5, 8, and 15 months. After implantation, an anteroposterior and lateral radiological study was carried out. Samples were sectioned at 5 μm and stained using hematoxylin-eosin, Masson's trichromic, and Gordon-Switt reticulin stains.*

Results: *These results confirmed the biocompatibility of this porcine biomaterial-collagen paste; only a few, occasional macrophages and scattered lymphocytes were observed. No fibrosis was observed between the implants and the bone. Moreover, the material was osteoconductive acting as a "scaffold" for bone cells, and there was a progressive increase in bone growth in and around the implants.*

Conclusion: *This new porcine biomaterial-collagen paste seemed to be biocompatible, bioresorbable, and osteoconductive. (Implant Dent 2012; 21:112–117)*

Key Words: biomaterial, porcine bone, xenografts, hydroxyapatite

The substitution of bone tissue constitutes an unsolved problem that requires new research into diverse materials (metals, polymers, and ceramics) capable of repairing defects and stimulating host bone growth to achieve repair. This problem has been partially resolved by the use of bone grafts; nonetheless, there is always a search of new, and possibly better, material.[1] Xenografts (obtained from other species) display osteoconductive properties only. This is the process by which the implanted material provides a scaffold for bone growth starting from the edge of the defects. The ridge-preservation approach using porcine bone in combination with collagen membrane significantly limited the resorption of hard tissue ridge after tooth extraction compared with extraction alone.[2] These materials seem to be slowly resorbable. The osteoconductive process implies that the material has the capacity to influence nonpluripotential cells to be converted into osteoblasts, which are involved in bone regeneration. This process differs from osteoinduction in that it occurs in predetermined cells and not in pluripotential cells, and as such the response to osteoconduction is restricted to an already programmed cell population.[3–8] The results of other studies showed that the mixture of autologous and equine bone was biocompatible, and its use was associated with new blood vessels ingrowth during healing, which has been found to be extremely important for bone formation.[9]

The ideal biomaterial must be biologically safe, and the safety of a bone substitute material depends on reliable reproducibility, biocompatibility, and absence of toxicity. Clearly, all aspects of a substitute must be studied thoroughly to make predicative risk assessment possible.[10] The different bone substitute products developed by the biomedical industry have had widespread clinical uptake and an analysis of the results of their use points to the overall superiority of bone substitutes of natural origin over derivative substitutes.[11] In particular,

*Senior Lecturer of General Dentistry, Faculty of Medicine and Dentistry, University of Murcia, Murcia, Spain.
†Professor Responsible of Pharmacological Interactions in Dentistry, Dental School, University of Granada, Granada, Spain.
‡Collaborate Professor of Pharmacological Interactions in Dentistry, Dental School, University of Granada, Granada, Spain.
§Senior Lecturer of Children Dentistry, Faculty of Medicine and Dentistry, University of Murcia, Murcia, Spain.
‖Full Professor of Oral Pathology and Medicine, Dental School, University of Chieti-Pescara, Chieti, Italy.
¶Associate Professor of Oral Surgery, Faculty of Medicine, University of Genova, Italy.
#Full Professor of Maxillofacial Surgery, School of Dentistry, Complutense University of Madrid; Head of the Department of Oral and Dental Implant Surgery, University Hospital of Madrid, Madrid, Spain.
**Orthopedic Surgeon, Unit of Cell Therapy & Bone Bioengineering, University Hospital V. Arrixaca, University of Murcia, Murcia, Spain.
††Collaborate Professor Faculty of Medicine and Dentistry, University of Murcia, Murcia, Spain.
‡‡Associate Professor of Oral Surgery, Faculty of Dentistry, Complutense University of Madrid, Madrid, Spain.

Reprint requests and correspondence to: José Luis Calvo Guirado, DDS, MS, PhD, Mozart 1, 1° G, E-30002 Murcia, Spain, Phone: 34-670785208, 34-968268353, Fax: 35-968268353, E-mail: josecalvog@gmail.com

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there is 1 animal species with a genotype close to human—the pig—and xenograft materials of porcine origin have provoked a great deal of research to assess their potential as a substitute for osseous grafts.[12] Various studies have shown that such materials provide an effective biocompatible, bioabsorbable, and osteoconductive matrix.[13–17]

In our recent study, we suggests that OsteoBiol mp3 (Tecnoss, Coazze, Italy), antigen-free bone consisting of 90% granules of between 600 and1000 μm mixed with 10% pure type I porcine collagen, may be a biocompatible material, causing only a minor, early-stage inflammatory response. It also has osteoconductive properties with the material acting as "scaffolding" for bone cells leading to progressive increases in bone growth in and around the xenograft. We also observed the substitution of osteoid tissue by adipose and hematopoietic bone marrow, a process that points to the partial and progressive resorption capacity of this collagenized porcine xenograft material. It may be considered a satisfactory substitute for bone tissue, a material that does not interfere with the bone's normal reparative processes.[18]

Aim of this study was to evaluate the bone response to a porcine xenograft made up of 80% corticocancellous collagenated bone particles of $\leq$300 μm in size, in the form of a bone paste (OsteoBiol Putty), placed in tibial bone defects in rabbits.

MATERIALS AND METHODS

Animals, Surgery, and Treatment

A total of 40 OsteoBiol Putty implants were placed in the proximal metaphyseal area of both tibiae of 20 albino New Zealand rabbits (30–35 weeks of age and weighing 3900–4500 g). All experiments were approved and performed according to the Spanish Government Guide and European Community Guide for animal care. Fifteen minutes before general anesthesia, the animals received an intramuscular injection of 0.5 to 1 mg/kg acepromazine maleate, an anxiolytic. General anesthesia included ketamine plus chlorbutol, 5 to 8 mg/kg intravenously; 0.5 to 1 mg/kg acepromazine maleate as coadjuvant; and 0.05 mg/kg atropine. Amoxicillin (0.1 mL/kg intramuscularly) was administrated at the end of surgery.

The internal approach was performed in the proximal metaphyseal-diaphyseal area of the tibia, 5 mm below the anterior tibial tuberosity, the removal of bone tissue to form concave defects was performed by a 4 mm diameter trephine drill at low rotation and constant irrigation. These were then filled with the paste-like xenograft. The 5 rabbits from each group were killed with an intracardiac overdose of thiopental: at 1 month (group 1), 5 months (group II), 8 months (group III), and 15 months (group IV) after implantation.

Radiological Study

Two x-rays (anteroposterior and lateral) of the section of bone containing implants were taken using the MAMMO-DIAGNOST UC (Philips, Madrid, Spain) mammography technique with Min-R Screen film (Kodak, Madrid, Spain), with the initial coarse-grained x-rays taken at 32 kV and 40 mA, using automatic exposimetry, with subsequent fine-grained images taken at 1:1.7, 20 kV and 16 mA, using automatic exposimetry, without the antidiaphragm grid.

Optical Microscopy

The surgically acquired samples were fixed in 10% neutral-buffered formalin and decalcified by 2 methods: the traditional decalcification method using 10% acid, by the TBD-I technique (Thermo-Shandon SA, Pittsburg, PA) for 2 hours (14% hydrochloric acid and polyvinylpyrrolidone); and the TBD-II technique (26% formic acid + 8.5% sodium citrate + polyvinylpyrrolidone) for 17 days, renewing the solution every 24 hours. Subsequently, all the samples were included in paraffin by the usual method, sectioned at 5 μm and stained using hematoxylin-eosin, Masson's trichromic, and Gordon-Switt reticulin stains. The most useful and convenient stain method was the hematoxylin-eosin whereas the Masson trichromic stain was used to reveal the newly formed lamellar bone and the Gordon-Switt stain showed the bone maturation. All samples were examined under light microscopy (Microphoto FXA, Nikon, Tokyo, Japan).

The entire circumference of the section (containing bone, grafted particles, and connective tissue [CT]) was traced manually to create an individual region of interest, in which the relative area of bone tissue (stained purple) and residual particles (stained black) was measured as percentage of the total region of interest area.

Morphometrical Analysis

The histomorphometrical evaluations comprised measurements of the area of bone and porcine particles in relationship with the total measurement area. Examinations were performed in a Nikon Eclipse 80i microscope (Teknooptik AB, Huddinge, Sweden) equipped with an EasyImage 2000 system (Teknooptik AB) using $\times$1.0 to $\times$40 objectives for descriptive evaluation and morphometrical measurements. Values for the total percentage of new bone, porcine bone biomaterial remaining graft, and nonmineralized CT were then calculated.

Statistical Analysis

In a first step, factors such as individual difference and position of the implant could be excluded as not significant. Tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk) and equality of variances (Mann-Whitney and Wilcoxon) were applied without any observed violation assumptions. The analysis of variance test, a parametric test, was used to identify the signifi-

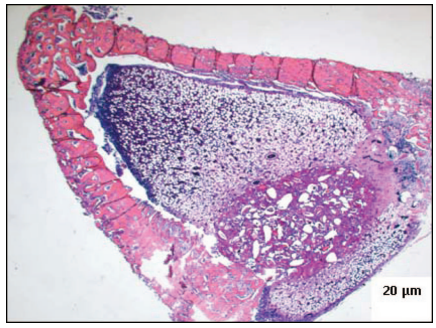

Fig. 1. Detailed image of the implant with focal points of the porcine bone biomaterial, granular tissue, and newly formed trabeculae (H&E), 20-μm scale.

Table 1. Mean Values for Remaining New Bone, Porcine Bone Biomaterial, and Nonmineralized Connective Tissue of All the Groups

Groups	New Bone (%)	Biomaterial	Connective Tissue
Group I (1 mo)	20.7 ± 1.5	28.8 ± 3.1	50.5 ± 3.1
Group II (5 mo)	22.4 ± 2.4	26.5 ± 2.1	51.5 ± 2.2
Group III (8 mo)	29.9 ± 0.12	22.4 ± 2.3	47.7 ± 1.7
Group IV (15 mo)	36.2 ± 1.6	16.4 ± 1.7	47.4 ± 2.6
Mean ± SD	27.32 ± 1.4	23.52 ± 2.3	49.15 ± 2.4

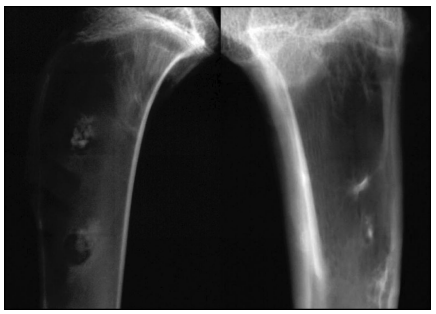

Fig. 2. Lateral x-ray of tibia (group 1) revealed the characteristics of the implanted material as being a cylindrical element, with a 4 × 6 mm rectangular structure, whose radiological density was greater than that of calcium, which allowed it to be identified within the trabecular bone structure in which it was implanted.

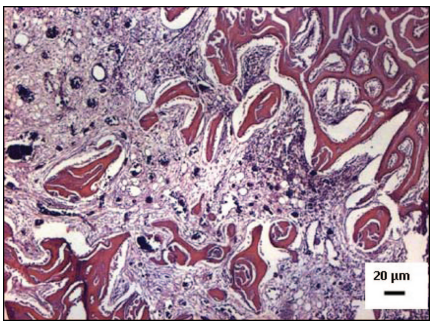

Fig. 3. Capillary blood vessels, and abundant mesenchymal cells of an irregular morphology with ample cytoplasm and numerous fibroblasts arranged randomly in a matrix of abundant fundamental substance, collagen fibers, macrophages, and scattered lymphocytes (H&E), 20-µm scale.

cance of the mean differences of bone to implant contact and standard deviations using a specific software (SPSS version 15.0 for Windows, Chicago, IL). The mean values and standard deviations among animals were calculated for each variable.

For the analysis, the central portion of each core was selected to avoid any potential bias and both the coronal (native host's remaining bone) and the apical portion (using a safe margin of 1.5–2 mm) were excluded from the analysis (Fig. 1).

RESULTS

Table 1 showed the mean values of the new bone, porcine bone biomaterial, and nonmineralized CT of all the groups were 27.32% ± 1.4%, 23.52% ± 2.3%, and 49.15% ± 2.4%, respectively, at 15 months. No statistical significant differences between all the groups were found.

Results at 1 Month

X-rays revealed the characteristics of the implanted material as being a cylindrical element, with a 4 × 6 mm rectangular structure, whose radiological density was greater than that of calcium, which allowed it to be identified within the trabecular bone structure in which it was implanted (Fig. 2). Optical microscopy of the implant and cortical defect site revealed the substitution of bone by granulated tissue extending toward the implant xenograft and invading the implanted material, which it partially furnished. This tissue was made up of numerous endothelial yemas and capillary blood vessels, and abundant mesenchymal cells of an irregular morphology with ample cytoplasm and numerous fibroblasts arranged randomly in a matrix of abundant fundamental substance, collagen fibers, macrophages, and scattered lymphocytes (Fig. 3).

Results at 5 Months

X-rays highlighted the cortical-osteoblastic line as being completely repaired, albeit with less density than that of the adjacent cortical bone. The radiological density of this material was lower than that observed for the

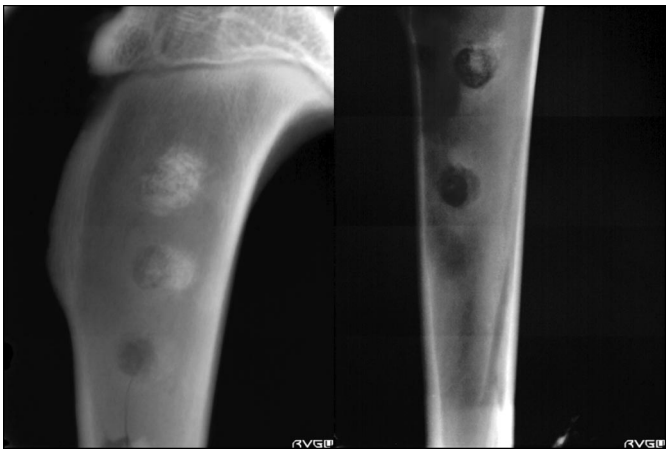

Fig. 4. Lateral x-ray of tibia (group II). Biomaterial implant with regular borders (white) inside the tibia. The cortical bone is almost being completely repaired, albeit with less density than that of the adjacent cortical bone (group II). The radiological density of this material was lower than that observed for the previous time period, although a reinforced radiological density could imply the formation of bone around the implant.

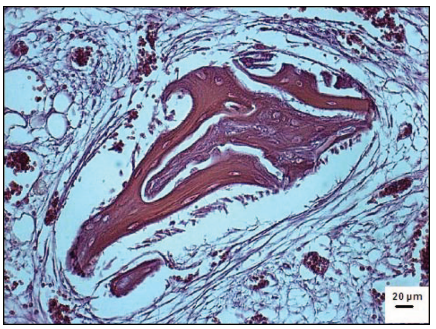

Fig. 5. The implanted xenograft had been substituted by osseous trabeculae, which were more extensive and thicker than those observed for the previous time period, and thus awarding a reticular appearance to the implant zone. No inflammatory response was observed (H&E), 20-µm scale.

previous time period, although a reinforced radiological density could imply the formation of bone around the xenograft implant (Fig. 4). The spherical form of the implanted material gave way to a more oval and irregular one. The pathological study highlighted the repair of the cortical bone as being almost completed by newly formed or immature bone tissue. Likewise, the implanted xenograft had been substituted by osseous trabeculae, which were more extensive and thicker than those observed for the previous time period, and thus awarding a reticular appearance to the implant zone (Fig. 5). No inflammatory response of note was observed.

Results at 8 Months

X-rays revealed that the external cortex of the artificial bone defects, in which the bone-putty implant had been introduced, had a calcium density similar to that of the adjacent cortex, and it was difficult to determine the entrance orifice (Fig. 6). On a cortical level, the xenograft implant displayed a decreased radiological density with respect to the previous group, and a more oval shape with a lower calcium density within. No radiologically well-defined borders were distinguishable. In some areas, there was continuity between the osseous cortex and the implanted material as seen by the linear image of osseous trabeculae. The pathological study highlighted the complete bone repair of the cortex at the implant orifice, by well-organized trabecular bone at the cortex with an increase in bone remodeling (Fig. 7). The formation of bone trabeculae and a marked increase in hematopoietic and adipose bone marrow in the center, which had partially replaced the granulated tissues, was observed, albeit to a lesser degree.

Results at 15 Months

X-rays of the bone defects in "controls" at the end of the experiment revealed the radiological aspect of the bone as being similar to that described for the previous group, with one or more rectilinear lines of a calcium density, which were still observable perpendicularly traversing the bone where the rectangular-shaped osseous defect had been artificially produced (Fig. 8). The bone defects into which the xenografts had been placed displayed the radiological image of an undefined geometric structure, with a decrease in graft volume and the complete repair of the osseous defect also being observed. Radiological images showed those trabeculae reaching the implant as being greater in number and density than those of the previous time periods, giving the implanted area a slightly reticular appearance. No healed or residual bone alterations attributable to the presence of the implant were observed. No structural changes in bone development over the study period were observed. In this last period, the pathological study was characterized by the presence of mature bone in the cortex at the implant insertion site, such that it was not different from the adjacent cortex. We also observed the remodeling of bone trabeculae around the implants, which was more pronounced in the proximity of the cortex (Fig. 9).

The morphometrical measurements revealed an increased amount of mineralized bone with time with significant differences between the different time periods. In parallel, a similar decrease of the area occupied by the grafted material was observed in all the groups. No statistical differences were found regarding the resorption of the grafted material in the different groups.

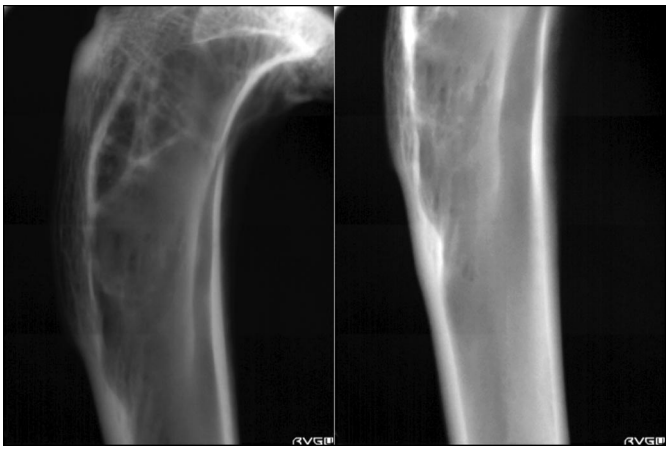

Fig. 6. Lateral x-ray of tibia (group III). X-rays revealed the external cortex of the artificial bone defects, in which the bone-putty implant was introduced, as having a calcium density similar to that of the adjacent cortex. It is thus difficult to determine the entrance orifice. The implant showed a decreased radiological density with respect to the previous group, and a more oval shape with a lower calcium density.

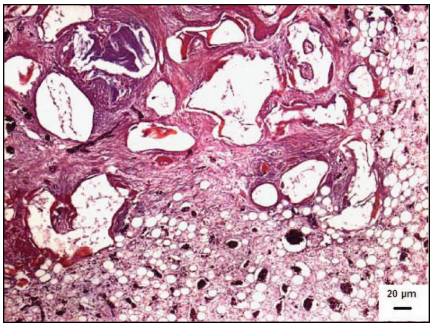

Fig. 7. Complete bone repair of the cortex by well-organized trabecular bone at the cortex with an increase in bone remodeling. The formation of bone trabeculae, and a marked increase in hematopoietic and adipose bone marrow in the center, which had partially replaced the granulated tissues (H&E), 20-μm scale.

DISCUSSION

Nitric formalin has traditionally been used in bone decalcification techniques; recently, the use of 2 commercial solutions known as TBD-1, which contains 14% hypochloric acid and polyvinylpyrrolidone, and TBD-2 (26% formic acid + 8.5% sodium citrate + polyvinylpyrrolidone) has become more common and have now habitually been used for several years in clinical cases in a number of hospitals. In this study, both methods were used, providing higher quality images of morphological characteristics. Thus, despite the low number of bibliographical

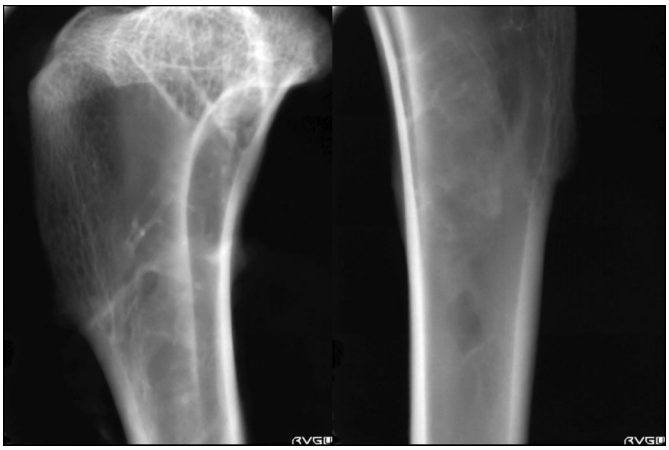

Fig. 8. Lateral x-ray of tibia (group IV). One or more lines of calcium density that were still observable perpendicularly traversing the bone where the rectangular-shaped bone defects had been artificially produced. Reticular appearance of the implant site with an absence of xenograft particles.

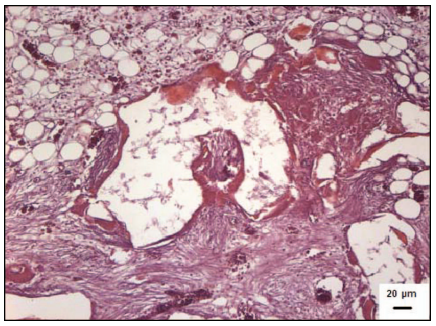

Fig. 9. Newly formed trabeculae bone without inflammatory cells. Remodeling of the trabecular bone around the implant was present, more pronounced in the proximity of the cortex (H&E), 20-μm scale.

references found in studies using TBD-1 and TBD-2,[19] we feel that TBD is a method which provides better results than those of nitric formalin for research into osseous tissues. Current implantology methods are achieving higher and higher success rates together with the ongoing development of implant systems; at the same time, the demand for treating complex cases requiring previous bone regeneration by the use of grafts is also rising. In this sense, autologous grafts continue to be the best option; however, many clinicians seek alternatives that avoid both secondary surgical zones (donor sites) and undesirable postoperative stages, and which also reduce surgical time.[20] Based on this premise, numerous studies demonstrate the effectiveness obtained with diverse bone-substitute biomaterials in sinus lift, ridge preservation, and atrophic maxilla procedures.[21–24]

The grafting material used in this study was OsteoBiol Putty, an antigen-free bone paste consisting of 80% corticocancellous collagenated granules of $\leq$300 μm mixed up of a heterodimer with 2 identical α-1 chains and one α-2 chain, and a homotrimer with 3 identical α-1 chains. The α-1 chains were in turn made up of 338 consecutive Gly-Xaa-Yaa triplets, where Gly is glycine, and Xaa and Yaa are different amino acids (excepting tryptophan and cysteine), these being proline and hydroxyproline, respectively, in 1 of every 3 cases. This biomaterial displayed a very good malleability and plasticity, thanks to it structure and characteristics, making it easy to apply and, moreover, it has low radiopacity. This experiment has confirmed the biocompatibility of OsteoBiol Putty because a scarce inflammatory response was observed only in the initial stages of the study, with a low number of scattered lymphocytes and macrophages, with no granuloma formation. Neither was any fibrosis observed on the border between the graft and the host bone which, together with the fact that bone growth was observed both around and within the implants, confirms the osteointegration capability of OsteoBiol Putty. The biocompatibility and osteointegration capability of other forms of hydroxyapatite have previously been defined in a range of studies by a number of authors.[25–27] These results have also demonstrated the osteoconductive capacity of OsteoBiol Putty, which acted as a scaffold for the bone cells, even with a granulometry of $\leq$300 μm. Thus, these results confirmed those reported by Klawitter et al[28] who made a comparison between pore size and granulometry and the quantity of newly formed bone tissue. Hydroxyapatites of a pore size $<$5 μm begin forming bone. In the case of hydroxyapatites of a pore size from 100 to 160 μm, a 17% degree of bone formation was observed, which increased progressively to 96% for pore sizes of $>$276 μm. They concluded that the pore size and granulometry should not be overly reduced, because both pore diameter and interporotic connections have a significant effect on the type and quantity of newly formed bone tissue.

The results obtained by other authors,[28–30] who felt that the existence of interporosity and osteoconductive capacity of the xenograft material facilitated bone growth within the biomaterials, were also confirmed. In fact, in this experiment, bone growth was observed not only in surface pores but also in pores is one of the main factors influencing osteoconduction within the grafted material.[30] In the same way, other studies of hydroxyapatites of differing degrees of porosity have reached the conclusion that hydroxyapatite of high porosity osteointegrates better in terms of newly formed bone compared with noninterconnected porous hydroxyapatite.[31]

X-rays from the last 2 time periods, 8 and 15 months, indicated an appreciable decrease in total volume, which together with the descriptions derived from optical microscopy indicating the replacement of osteoid tissue by adipose and hematopoietic bone marrow, demonstrate the existence of partial and progressive resorption phenomena of the bone putty, which becomes more accentuated as of month 5 after implantation. This characteristic has been described for hydroxyapatite by previous authors.[31–35]

CONCLUSION

Our results suggest that OsteoBiol Putty may act as both, a biocompatible

material by causing only a minor, early-stage inflammatory response, and an osteoconductive biomaterial by allowing the rapid formation of granulated tissue replaced by trabecular bone.

DISCLOSURE

The authors claim to have no financial interest in any company or any of the products mentioned in this article.

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